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Observations of the Effect of B. abortus Bang on the Weight of the Spleen of the Guinea Pig

C. P. Fitch and R. E. Lubbehusen
Division of Veterinary Medicine

UNIVERSITY FARM, ST. PAUL

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OBSERVATIONS OF THE EFFECT OF B. ABORTUS BANG ON THE WEIGHT OF THE SPLEEN OF THE GUINEA PIG

C. P. Fitch and R. E. Lubbehusen

The use of small animals has played an important rôle in the isolation, study, and ultimate identification of pathogenic organisms in investigations of human and animal disease. Many advancements in our knowledge of disease have depended largely upon the utilization of the guinea pig and rabbit as means of study. Such study embraces the observation of the direct effect of the disease process upon the laboratory animal during life; or the examination of postmortem lesions, both gross and microscopic, together with recovery of the disease-producing organism for subsequent identification.

In our investigational work of bovine infectious abortion, guinea pig inoculation has been an invaluable aid from the standpoint of both diagnosis of infection and isolation of the organism for study from a variety of sources. As *B. abortus* Bang infection in guinea pigs does not usually manifest itself in any striking manner during life, the ultimate diagnosis of such infection has depended thus far upon serologic tests and the isolation of the organism by the direct culture method.

Many investigators have described postmortem lesions which have been observed in known cases of *Abortus* Bang infection in guinea pigs. Fabyan(1), in his studies of the pathogenesis of *B. abortus* Bang, describes in detail the gross and microscopic pathological changes of various organs of guinea pigs so affected. Schroeder and Cotton(2) observed certain lesions in guinea pigs resulting from the injection of milk which in many instances were confused with tuberculosis, until bacteriologic examination showed the causative organism to be *B. abortus* Bang. Smillie(3) has recorded in some detail the postmortem lesions found in guinea pigs inoculated with abortion-infected material from a variety of sources, and concludes: "Inoculation of guinea pigs with *B. abortus* Bang is regularly associated with an enlarged congested spleen. Other less constant lesions affect the testicles, kidneys, liver and bones." Further "The spleen is the organ in which the bacteria are regularly present and in large numbers. Cultures must be made from it to insure success." Meyer, Shaw, and Fleischner(4), in their study of the comparative pathogenicity of *B. melitensis* and *B. abortus*, conclude in part: "Experiments extending over several years support the well known fact that *B. abortus* may produce in guinea pigs an interesting inoculation disease. However, the degree of tissue injury in the spleen and lymph nodes may vary considerably." A study of the

results reported by these and other investigators reveals a diversity of opinion as to the constancy of certain postmortem lesions in guinea pigs infected with *B. abortus* Bang, and suggests that if such postmortem examination is to be of any value in substantiating the results obtained by serologic and cultural methods, the following points may well be considered: (1) Does *B. abortus* Bang infection manifest itself by gross pathological changes in certain organs? (2) If so, are these changes constant enough to prove of any value as an aid in diagnosis? (3) Does the source of the abortion infected material have any influence upon the nature of the postmortem lesions? (4) Does the length of the period of inoculation influence the pathologic changes incident to the disease? It was in answer to these questions that our observations of the influence of abortion infection on the weight of the spleen of the guinea pig were instituted, as this organ in many instances gave gross evidence of pathologic involvment when infected with *B. abortus*.

The data recorded herewith represent our observations on the autopsy of 87 guinea pigs in five years of investigational work in bovine infectious abortion. Infection in these pigs has in each instance been proved by direct culture and serologic test. The material with which these pigs were infected represents a variety of sources—fetal and placental material, milk and colostrum, cultures, and miscellaneous. The guinea pigs used in our routine inoculation of various materials of which the 87 positively infected ones herein recorded represent only a small percentage, were taken from supply stock without special regard to either age, weight, or sex. All pigs used were those that had made normal growth gains and were in apparent health at the time of inoculation.

WEIGHING AND AUTOPSY TECHNIC

After inoculation, the procedure of which will be discussed in conjunction with a review of the various charts, the pigs were confined in cages and allowed to remain for a variable period, ranging from three weeks to two months depending upon the nature of the material injected. Before autopsy all food was withheld for a period of 12 to 15 hours in order that the influence of the ingesta on the body weight would be approximately the same in all individuals. The animals were carefully weighed and the weights recorded in grams. All pigs were anesthetized by chloroform in an enclosed chamber and killed by severing the jugular. During this operation the blood was collected in a sterile centrifuge tube corresponding in number to the pig from which it was obtained, and set aside at ice-box temperature to be tested later by means of the agglutination test for the presence of abortion infection. The carcasses were next stretched upon metal trays and the hair coat of the abdomen was moistened with 5% phenol preparatory for autopsy. The skin was

incised along the median line from the pubis to the cariniform cartilage of the sternum, at which points transverse incisions were made extending well toward the extremities. Dissection of the skin from the lateral abdominal and thoracic walls and reflexion of the entire muscular wall of these two cavities exposed the organs for observation. The spleen was removed by severing the gastro-spleenic omentum with sterile scissors and forceps. After removal, the spleen was transferred to a sterile petri dish and the omentum was removed as close to the hilus as possible without injury to the spleenic tissue. As the presence of varying amounts of this omentum would necessarily influence the weight of the spleen, great care was always taken that it was completely removed. After completing the further autopsy, the spleen was weighed on an analytical balance and the weight recorded in grams to the third decimal. After obtaining the spleen weight, the substance of the organ was next cultured by direct transfer to serum agar. The following data were thus obtained:

1. Body weight in grams.
2. Spleen weight in milligrams.
3. Blood for serologic tests to check infection with *B. abortus* Bang.
4. Direct culture of the spleenic tissue further to check *B. abortus* Bang infection.

The data obtained in the autopsy of 87 positively infected guinea pigs showed that the body weights of these pigs ranged from 298 to 917 grams. It at once became obvious that before any deductions could be drawn on comparative weights of spleens, the normal weight of this organ must be determined for varying body weights.

DETERMINATION OF THE NORMAL WEIGHT OF SPLEENS

For the mathematical determination of the normal weight of guinea pig spleens based upon the body weight, we are indebted to the work of Bessesen and Carlson(5). These investigators, after an extended study of 82 guinea pig autopsies and a careful comparison of the relationship in the weight of certain organs and the body weight, were able to derive simple mathematical formulas to fit each growth curve. These formulas applied to the determination of the normal spleen weight were as follows:

$y=ax-b-cx^2$	For body weight of
$a=.0032 \quad b=0.08 \quad c=0.000005$	100–300 grams
$y=ax+b+cx^2$	For body weight of
$a=0.00054 \quad b=0.205 \quad c=0.0000007$	300–1000 grams

y=Spleen weight in grams.
x=Body weight in grams.
a, b, and c are constants.

A comparison of the above method of computation with the spleen weights of many non-infected guinea pigs used in our work has shown it to be reasonably accurate, altho there are instances in which there is considerable variation, as these formulas are based primarily on average observations. However, their relative accuracy can not be questioned.

Having the body weight, "x", by direct measurement, we were enabled by the use of the above formulas to compute the normal spleen weight in each case. We then had a basis for comparison of the effect of *B. abortus* infection upon the weight of spleens.

PRESENTATION OF DATA

In order that the statistical data obtained might be presented in concise form, graphs have been made. The charts are plotted with the spleen weight as the ordinate and the gross body weight as the abscissa, with the exception of Chart VIII, in which the number of days of inoculation represent the abscissa. To facilitate a direct comparison of infected spleens and norms, the normal curve based on the gross body weight was plotted on each chart.

OBSERVATIONS

CHART I

Chart I is the graphic presentation of the variation in weight of the spleens of 87 guinea pigs infected with *B. abortus* Bang, without regard to source of material or number of days of inoculation. These weights range from 4.627 to 0.530 grams. In five individuals, or 5.7%, the weight of the infected spleens is below that of the computed norm. It is possible that the actual weight obtained in these instances represents the norm for these individuals, as none of them presents any marked difference from the computed norm curve.

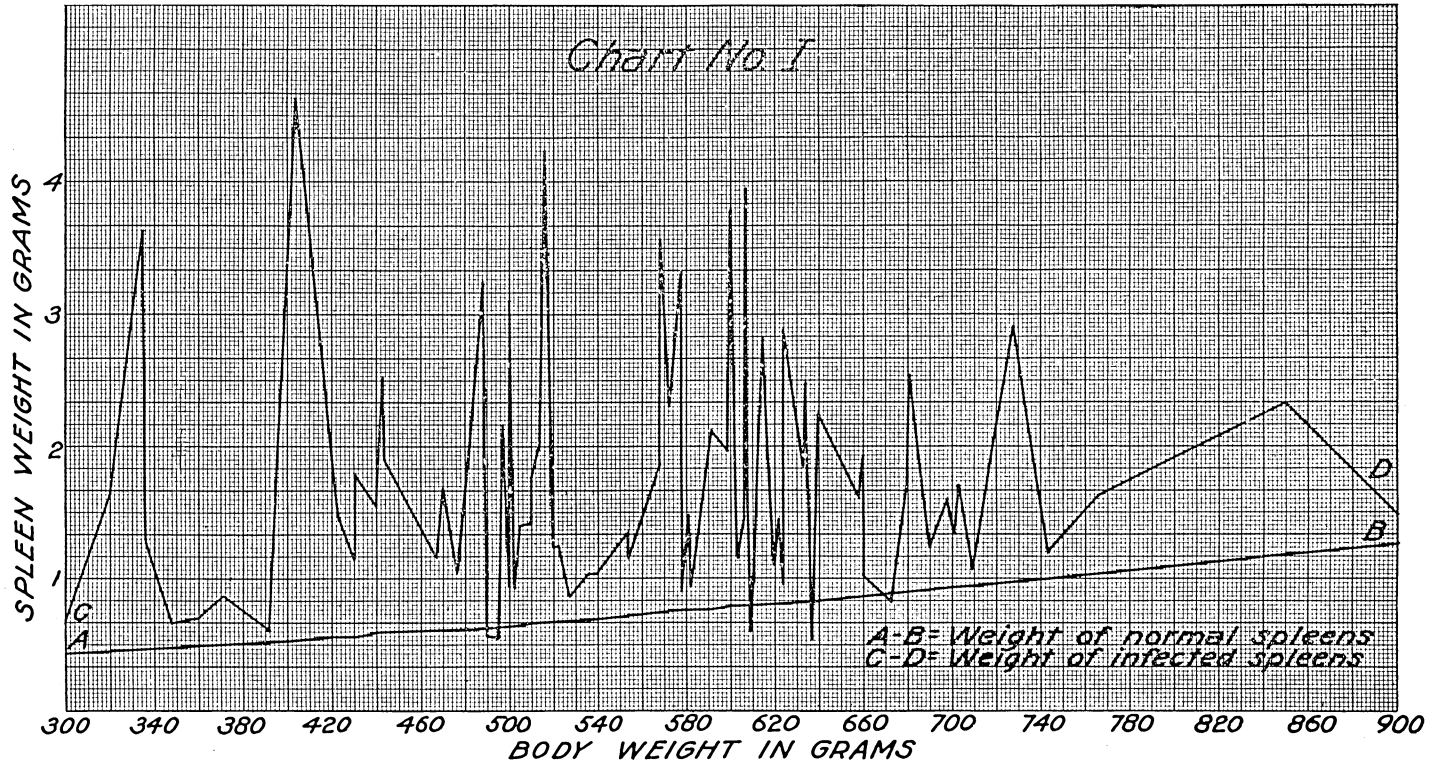
Chart No. 1
SPLEEN WEIGHT IN GRAMS
4
3
2
1
C
A
D
B
A-B= Weight of normal spleens
C-D= Weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700 740 780 820 860 900
BODY WEIGHT IN GRAMS

CHART II

Chart II represents the data of Chart I with the values plotted in the form of a mean curve. We find here that the average weight of all infected spleens is substantially above that of the norm curve, being 1.695 as compared to 0.734 grams for the norms.

These averages show that the weight of the spleen is materially increased in guinea pigs infected with *B. abortus* Bang. However, after a study of Chart I, the data of which present such a wide variation in weight of spleens, the following questions present themselves: (1) Does the source of the organism and possible virulence have any bearing upon the weight of the spleen? (2) Does the change in weight of the spleen bear any relation to the number of days of inoculation? Graphs were constructed on the basis of material injected and the number of days of inoculation in an effort to answer these questions.

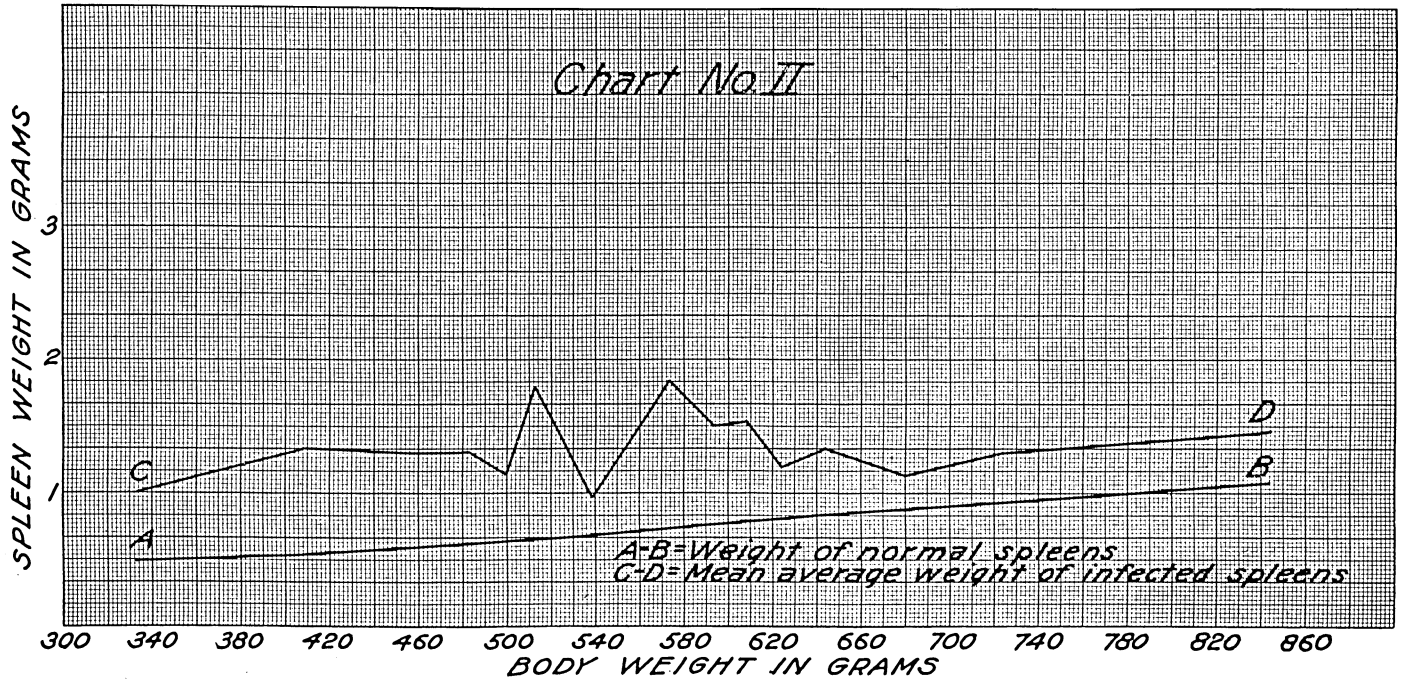
Chart No. II
SPLEEN WEIGHT IN GRAMS
3
2
1
C
A
D
B
A-B=Weight of normal spleens
C-D=Mean average weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700 740 780 820 860
BODY WEIGHT IN GRAMS

CHART III. FETAL MATERIAL

The inoculated material represented in this group consisted largely of fetal stomach contents. In the routine autopsy of feti, ligatures were placed at the esophageal and pyloric orifices of the stomach. After removal, a portion of the stomach wall was seared by means of a hot spatula and incised with a sterile sharp-pointed bistoury. The stomach contents were collected in a sterile container. When considerable amounts of mucous were present, it became necessary to dilute the material with physiologic saline solution before injection. The average amount of material injected ranged from 1½ to 3 cc., given intraperitoneally.

Thirteen guinea pigs having an average weight of 576.4 grams were injected with the above material. The average number of days of inoculation for the group was 42.5. An analysis of the data of Chart III shows that material from the same general source gives rise to a variation in spleen weight ranging from 4.627 to 0.572 grams, with an average of 2.241 grams for the group. The spleenic norm of the 13 pigs used, computed on the basis of average body weight, is equal to 0.738 grams. We find therefore that the average weight of infected spleens in this group has been increased approximately three times that of the normal.

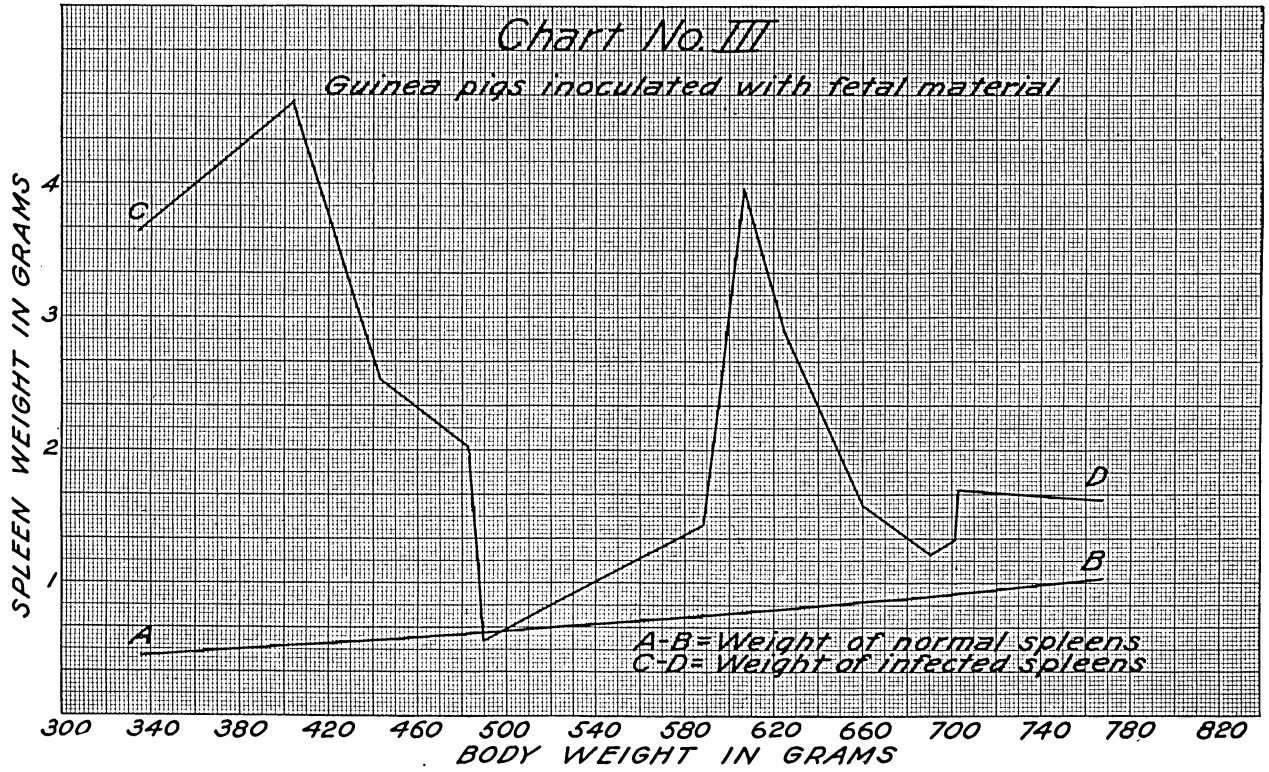
Chart No. III
Guinea pigs inoculated with fetal material
SPLEEN WEIGHT IN GRAMS
1
2
3
4
C
D
B
A
A-B = Weight of normal spleens
C-D = Weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700 740 780 820
BODY WEIGHT IN GRAMS

CHART IV. PLACENTAL MATERIAL

Immediately upon the removal of placental tissue, the chorion was examined and portions of the numerous tufts showing gross evidence of pathological change were removed. The material thus obtained was placed in a sterile mortar and thoroly triturated with the addition of physiologic saline. From ½ to 1 cc. of this saline suspension of placental tissue was injected intra-peritoneally.

Nine guinea pigs having an average body weight of 550.6 grams were infected by this means. The average period of inoculation for this group was 43 days.

It will be observed that the weights of infected spleens in this group with one exception range between 1 and 2 grams with an average of 1.776 grams, while the norm computed on the basis of the average body weight for the group of nine is 0.7237 grams. This increase is found to be somewhat less than that shown in Chart III.

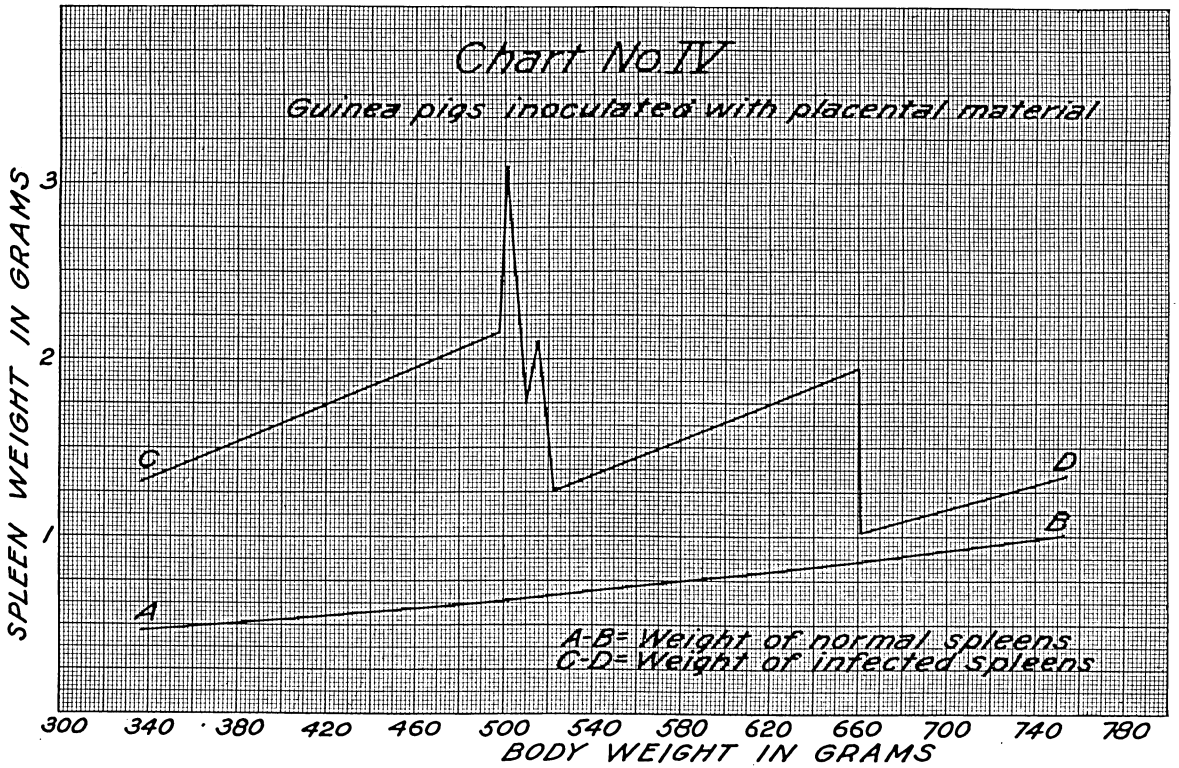
Chart No IV
Guinea pigs inoculated with placental material
SPLEEN WEIGHT IN GRAMS
3
2
1
C
D
B
A
A-B= Weight of normal spleens
C-D= Weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700 740 780
BODY WEIGHT IN GRAMS

CHART V. MILK AND COLOSTRUM

It has been our practice in studies in infectious abortion to make routine guinea pig inoculations with colostrum and milk from animals known to be infected with the Bang germ in order to determine its presence in these fluids. For this purpose approximately 1000 cc. of milk is obtained in a sterile flask from the four quarters of the udder and transferred to a sterile separatory funnel, where it is allowed to remain for 18 to 24 hours. At the end of this period samples of the milk are drawn into two 100-cc. centrifuge tubes and centrifuged for one and one half hours. The supernatant fluid is then removed and the sediment injected into a guinea pig, the amount ranging from 3 to 5 cc. At the same time a 5-cc. sample of whole non-centrifuged milk from the same cow is injected into a second pig. All inoculations are made intra-peritoneally.

The data of Chart V represent 39 infected guinea pigs of an average weight of 548.5 grams. The average period of inoculation was 42.2 days. While there appears to be a rather wide range in the weight of infected spleens, yet we find that only 7 of the entire 39 spleens show a weight exceeding 2 grams with an average of 1.492 grams for the group. The computed norm spleen weight based on the average for the group is 0.7340 grams.

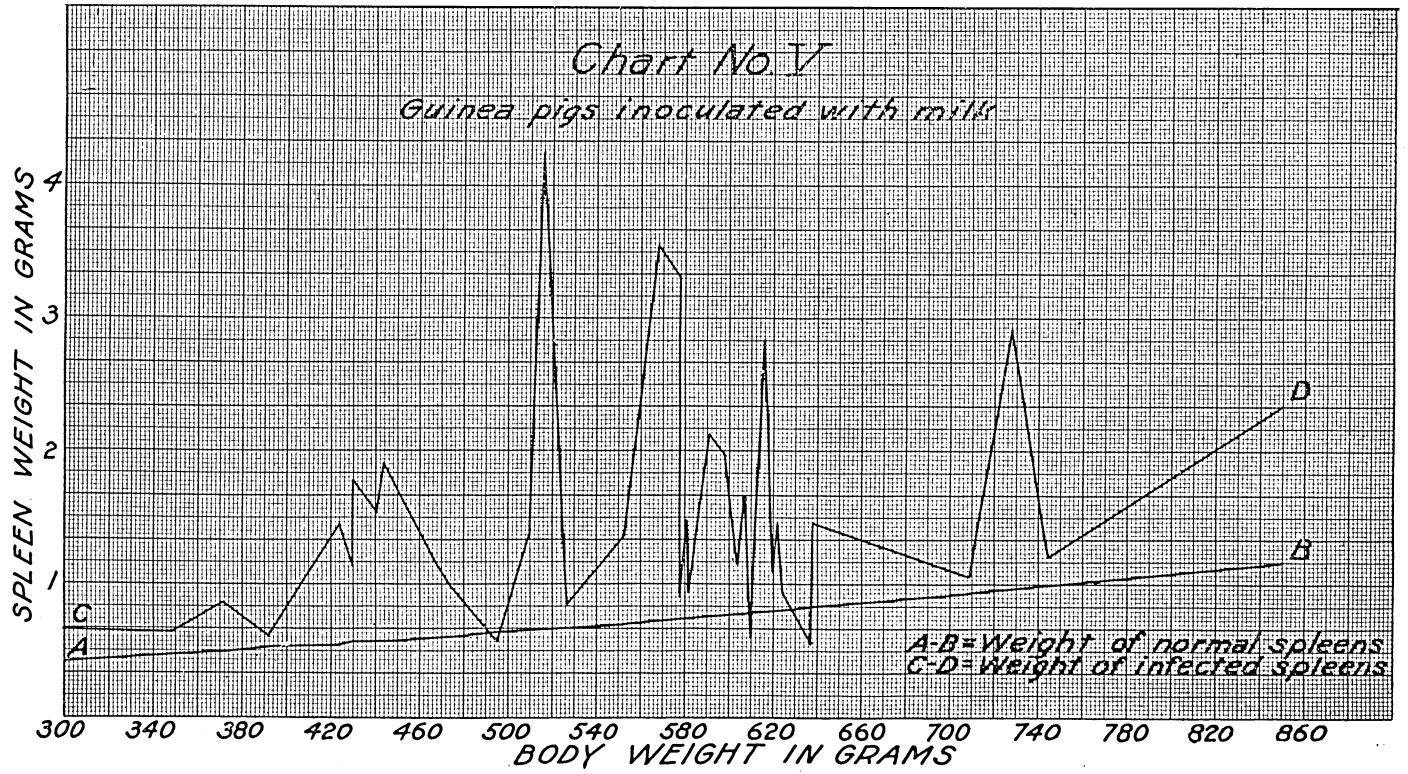
Chart No. V
Guinea pigs inoculated with milk
SPLEEN WEIGHT IN GRAMS
4
3
2
1
C
A
D
B
A-B = Weight of normal spleens
C-D = Weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700 740 780 820 860
BODY WEIGHT IN GRAMS

CHART VI. *B. ABORTUS* CULTURES

The infective material of Chart VI is represented in part by cultures which were obtained from suspected abortion infected material and passed through guinea pigs for purposes of isolation of the Bang organism; also by stock abortion cultures for purposes of study of various properties.

Seventeen guinea pigs having an average weight of 547 grams were infected by this means. The average period of inoculation was 43.7 days.

As may be expected when the nature of the infective material and the varying amounts injected are considered, the infected spleen weights vary to a considerable degree, namely from 0.564 to 3.788 grams, with an average of 1.815 grams. The average norm spleen weight for the group is 0.7093 grams.

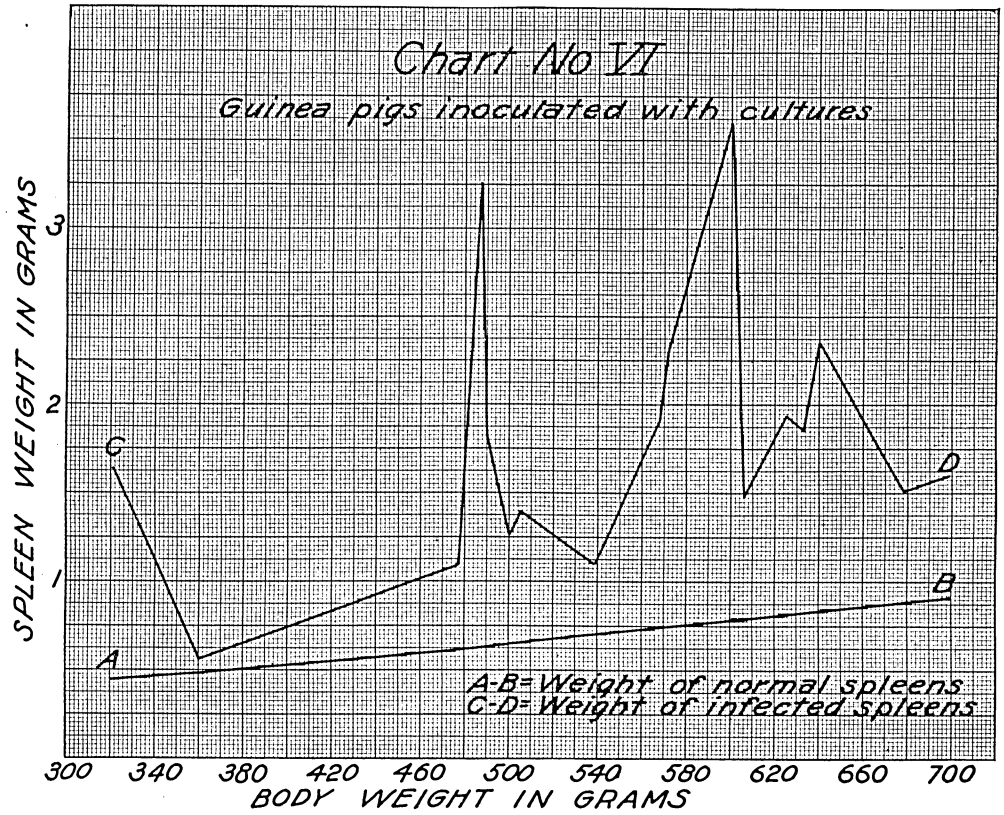
Chart No VI
Guinea pigs inoculated with cultures
SPLEEN WEIGHT IN GRAMS
3
2
1
C
D
B
A
A-B= Weight of normal spleens
C-D= Weight of infected spleens
300 340 380 420 460 500 540 580 620 660 700
BODY WEIGHT IN GRAMS

CHART VII. MISCELLANEOUS

Infective substances represented in Chart VIII are made up of miscellaneous materials encountered in our routine examination of suspected abortion in diagnosis work and not included in the graphs previously considered.

The abscess material was obtained from cases in which living abortion cultures had been used for vaccination purposes, and in which it was found necessary to drain the abscesses which sometimes resulted from this procedure. It may be observed that of the 9 guinea pigs infected with miscellaneous material, the 3 inoculated with abscess material show much higher spleenic weights than the others. This may possibly be explained on the basis of virulence of the organism. With the exception of that of one pig injected with vaginal discharge, the remaining 5 spleens show very little variation in weight.

The average weight of the 9 spleens represented is 1.505 grams, as compared with the computed norm of 0.7378 grams based on an average body of weight of 568.2 grams. The average period of inoculation for the group was 35 days.

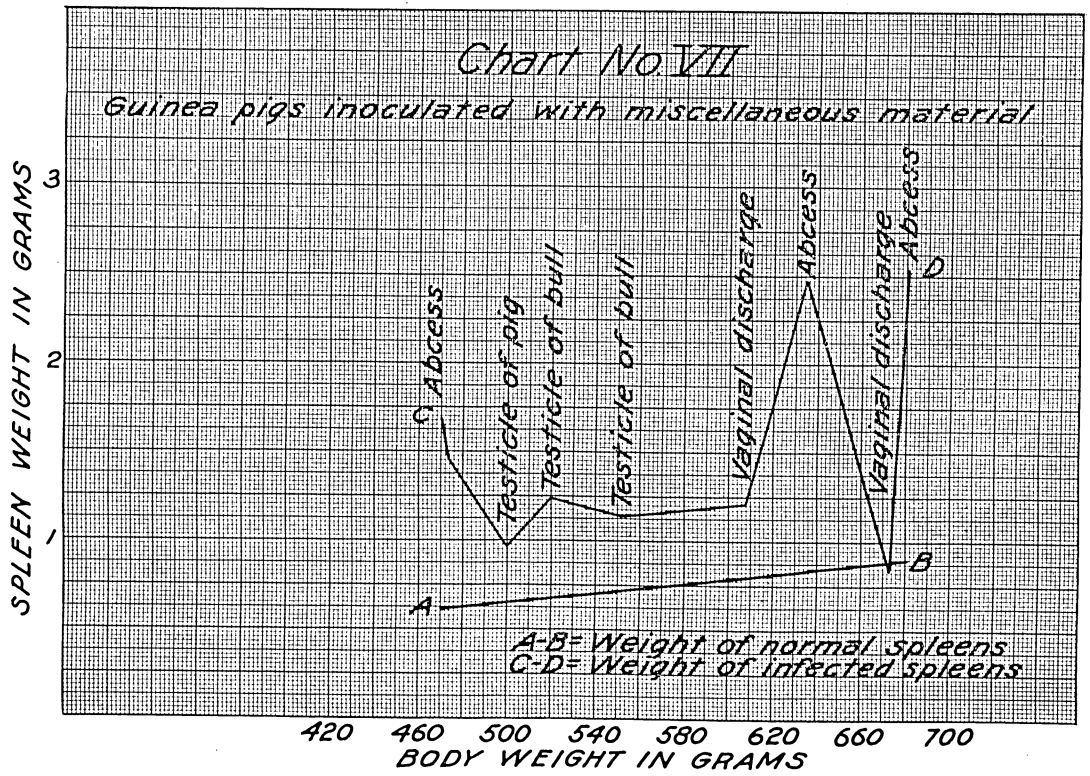
Chart No. VII
Guinea pigs inoculated with miscellaneous material
SPLEEN WEIGHT IN GRAMS
3
2
1
C Abcess
Testicle of pig
Testicle of bull
Testicle of bull
Vaginal discharge
Abcess
Vaginal discharge
Abcess
D
B
A
A-B= Weight of normal spleens
C-D= Weight of infected spleens
420
460
500
540
580
620
660
700
BODY WEIGHT IN GRAMS

CHART VIII. PERIOD OF INOCULATION

Chart VIII was prepared in order to determine the effect of the duration of time following inoculation on the weight of spleen. This graph was constructed with the abscissa representing the number of days of inoculation, and the ordinate, the spleen weight. The normal spleen weight was computed on the average body weight of the pigs inoculated for a definite period. For example, 3 pigs having an average body weight of 454 grams with a computed spleen norm of 0.5944 grams, and an average infected spleen weight of 3.069 grams were inoculated for a period of 28 days. No effort was made to classify the infected animals on a basis of materials used, as this phase of the question had already been considered. From the above example it will be noted that as the graph values represent the averages of different numbers of individuals, such values are only comparable to a relative degree, and in a sense are proportional to the number of animals considered. If the extremes as represented were to be eliminated when only one or two were considered per given period, the values would be regular in character and fall within comparatively narrow limits. After considering these various factors, we may conclude with a fair degree of accuracy that after 24 days (the minimum represented) the length of the period of inoculation is not proportional to and does not materially affect the spleenic weight.

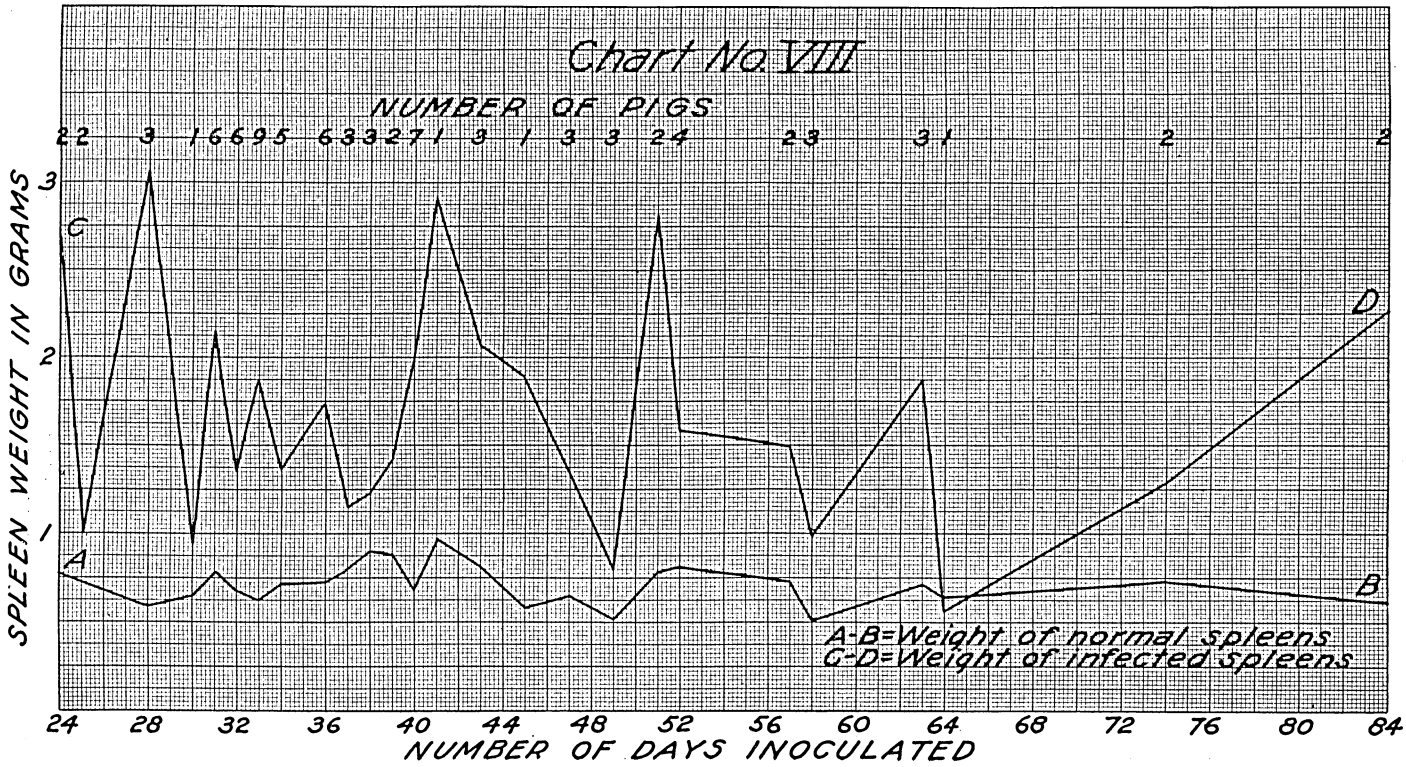
Chart No. VIII
NUMBER OF PIGS
2 2 3 1 6 6 9 5 6 3 3 2 7 1 3 1 3 3 2 4 2 3 3 1 2 2
SPLEEN WEIGHT IN GRAMS
3
2
1
C
D
A
B
A-B=Weight of normal spleens
C-D=Weight of infected spleens
24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84
NUMBER OF DAYS INOCULATED

SUMMARY

In order that the data obtained from our observations on the influence of various abortion infected materials may be compared, the following table is presented:

Material	No. of pigs inoculated	Av. weight of pigs	Av. norm. spleen weight	Av. infected spleen weight	Av. No. of days of inoculation
Fetal	13	576.4	0.7488	2.241	42.5
Placenta	9	550.6	0.7145	1.776	43.0
Milk	39	548.5	0.7117	1.492	42.2
Cultures	17	547.0	0.7108	1.815	43.7
Miscellaneous	9	568.2	0.7378	1.505	35.0
	87	558.8	0.7247	1.695	41.3

We find that the average body weight and consequently the computed spleenic norm of the guinea pigs used for each group are not variable to any extent and therefore compare quite closely to the average for the entire 87 pigs. Again it will be observed that with the exception of the miscellaneous group the length of the period of inoculation is very nearly equal, and even if we include the miscellaneous group, the average thus obtained does not differ materially from that of any one group. Therefore as the weights of the pigs, spleenic norms, and length of period of inoculation compare very closely in these various groups, we are justified in making a direct comparison of the infected spleen weights, as these represent the average of each group.

While these observations indicate that there is an increase in the spleen weight of pigs infected with *B. abortus* Bang, yet considering that various other factors may give rise to spleenomeglia, such an increase alone can not be considered of diagnostic value without the actual demonstration of the organism. While it is true that in spleenomeglia due to abortion infection there are gross and microscopic pathologic changes that are quite characteristic, it is likewise true that *B. abortus* Bang may be recovered from spleens showing little or no gross change. It is because of these exceptions to the average that we can not place too much reliance upon the use of the spleen weight as an aid in the diagnosis of *B. abortus* Bang infection.

CONCLUSIONS

1. *B. abortus* Bang infection in guinea pigs may and usually does give rise to a marked increase in spleen weight.

2. Infective materials from various sources seem to affect the spleen weight in varying degrees.

3. After the first 24 days (minimum considered) the length of the period of inoculation bears no relation to the increase in the weight of the spleen.

4. *B. abortus* Bang may be isolated from spleens which show no increase in weight or gross evidence of pathological involvement.

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